COMPOSITIONS FOR CELL-BASED THERAPIES AND RELATED METHODS

CLAIM OF PRIORITY

This application claims priority to U.S. Provisional Application No. 63/273,678, filed October 29, 2021. The entire disclosure of the foregoing application is incorporated by reference in its entirety.

BACKGROUND

Treating chronic and genetic diseases by implanting living cells that produce a therapeutic substance capable of treating such diseases has exciting potential to improve the health of patients with such diseases. To fully achieve this potential, the implanted cells must be protected from the patient's immune response, so that they remain viable, and the implanted cells must also be capable of producing therapeutic levels of the desired therapeutic substance for several weeks, months or even longer. One exploratory approach for delivering such cell-based therapies is to encapsulate the therapeutic-producing cells in alginate hydrogel capsules, with the objective that the capsule structure isolates the therapeutic-producing cells from immune system cells, while allowing entry of nutrients for the therapeutic-producing cells and exit of the therapeutic substance from the capsules. Once such hydrogel capsules are produced, they need to be stored in a manner that preserves the integrity of the capsule structure and viability of the encapsulated cells.

SUMMARY

In one aspect, the present disclosure provides a composition comprising a population of hydrogel capsules disposed in a pharmaceutically acceptable aqueous solution, wherein each hydrogel capsule in the population encapsulates a plurality of live mammalian cells, wherein the solution has a pH of between 6.0 and 9.0 at 12˚C to 30˚C (e.g., about 15-25˚C), and comprises a calcium salt at an elemental calcium concentration of between about 1.0 millimolar (mM) and about 10 mM.

In one example, the present disclosure provides a hydrogel capsule composition, which comprises a population of hydrogel capsules and a pharmaceutically acceptable solution, wherein each hydrogel capsule in the population comprises an ionically cross-linked alginate and encapsulates a plurality of live mammalian cells. In an example, the solution comprises a calcium salt at an elemental calcium concentration of between about 1.0 mM and about 10 mM. In an example, the elemental calcium concentration is between about

1.2 mM and about 3 mM. In an example, the calcium salt is calcium chloride. In an example, the solution has an osmolality of about 250 mOsm/kg to about 350 mOsm/kg and a pH of between 6.0 and 9.0 at a temperature of 12˚C to 30˚C.

In some examples, the solution further comprises at least one buffering agent capable of maintaining the pH of the solution within a desired range (e.g., between 6.0 and 9.0) when the composition is stored at a temperature of 12˚C to 30˚C (e.g., about 15-25˚C). In an example,

the buffering agent comprises one or more of an acetate salt (e.g., sodium acetate), a gluconate salt (e.g., sodium gluconate), a bicarbonate salt (e.g., sodium bicarbonate), a lactate salt (e.g., sodium lactate), or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES).

In an example, the solution further comprises a carbon source (e.g., a sugar (e.g., dextrose, glucose, galactose, hexose, fructose, maltose), glycerol, glutamine, pyruvate) that supports viability of the encapsulated cells. In an example, the carbon source is glucose. In an example, the solution comprises about 1.5 mM to 2.5 mM calcium chloride, about 5 mM to about 25 mM D-glucose, and about 40 mM to about 50 mM sodium bicarbonate.

In some examples, the solution further comprises minerals, amino acids and vitamins that support viability of the encapsulated cells.

In an example, the minerals include a magnesium salt (e.g., magnesium chloride or magnesium sulfate) or a potassium salt (e.g., potassium chloride).

In an example, the amino acids include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

In an example, the vitamins include a vitamin B1 compound (e.g., thiamine or a thiamine salt, e.g., thiamine hydrochloride), a vitamin B3 compound (e.g., nicotinic acid or niacinamide) and a vitamin B6 compound (e.g., pyroxidine or a pyroxidine salt, e.g., pyroxidine hydrochloride).

In some examples, the amino acids include L-arginine, L-cystine, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

In some examples, the vitamins include choline or a choline salt (e.g., choline chloride), a vitamin B1 compound (e.g., thiamine or a thiamine salt, e.g., thiamine hydrochloride), a vitamin B3 compound (e.g., nicotinic acid or niacinamide), a vitamin B5 compound (e.g., pantotheneic acid or calcium pantothenate), a vitamin B6 compound (e.g., pyroxidine or a pyroxidine salt, e.g., pyroxidine hydrochloride), a folate compound (e.g., folic acid), riboflavin and i-inositol.

In an example, the hydrogel capsules in the hydrogel capsule population are sphere-like or spherical in shape. In an example, the hydrogel capsules have a mean capsule diameter of about 500 μm to about 5000 μm (e.g., about 500 μm to about 4000 μm, about 500 μm to about 3000 μm, about 500 μm to about 2500 μm, about 500 μm to about 2000 μm, about 500 μm to

about 1500 μm, about 500 μm to about 1000 μm, about 1000 μm to about 2500 μm). In an example, the hydrogel capsules are not sphere-like or spherical in shape. In an example, each capsule comprises a cell-containing compartment surrounded by a barrier compartment which comprises the ionically cross-linked alginate. In an example, the cross-linked alginate comprises an alginate covalently modified with at least one afibrotic compound, as defined herein. In an example, the cross-linking agent comprises barium ions. In some examples, the cell containing compartment encapsulates the live mammalian cells in a first polymer composition comprising an alginate, which is optionally ionically cross-linked (e.g., with barium ions). In some examples, the alginate in the first polymer composition is covalently modified with a cell-contacting peptide, as defined herein. In an example, the mean capsule diameter of the hydrogel capsules is 1400 to 2000 μm.

In some example, the encapsulated live mammalian cells are derived from a human cell, e.g., from an RPE cell (e.g., an ARPE-19 cell). In an example, the cells are genetically modified to express and secrete an exogenous protein, e.g., any of the therapeutic proteins described herein.

In another aspect, the present disclosure provides a sealed container comprising any of the capsule compositions described herein. Each interior surface of the container in contact with the composition consists essentially of a biocompatible material, e.g., a medical grade plastic (e.g., fluorinated ethylene propylene (FEP) or polyethylene terephthalate glycol (PETG)). In an example, the bottom interior surface of the container has a rectangular or round shape.

In an example, substantially all (e.g., at least 90%, 95%, 98% or greater) of the hydrogel capsules in the composition are distributed substantially uniformly across the bottom interior surface of the container in a capsule layer and a volume of the solution (VS) is disposed on top of the layer. In an example, the capsule layer has a depth equal to about 1.00 to about 1.25 times the average diameter of the capsules in the composition.

In another aspect, the present disclosure provides a use of a composition as described herein in the manufacture of a medicament for treating a subject in need of a therapeutic substance, wherein a therapeutically effective amount of the composition is to be administered to the subject, wherein the subject is human, and wherein the administration comprises the effective amount to be disposed into the intraperitoneal cavity of the subject.

In another aspect, the present disclosure provides a method of making a sealed container comprising a hydrogel capsule composition, wherein the method comprises:

(i) providing a population of hydrogel capsules encapsulating live mammalian cells, optionally wherein the mammalian cells are genetically modified to express and secrete a therapeutic substance, e.g., a therapeutic polypeptide;

(ii) combining the population of hydrogel capsules with a pharmaceutically acceptable aqueous solution;

(iii) placing a desired volume of the capsule composition into a biocompatible, sealable container in a manner that produces a capsule layer in which substantially all of the capsules in the composition volume are distributed substantially uniformly across the bottom of the container at a depth equal to about 1.00 to about 1.25 times the mean diameter of the capsules in the composition; and

(iv) sealing the container.

In yet another example, the present disclosure features a method of making a sealed container comprising a hydrogel capsule composition described herein. In an example, the method comprises providing a population of hydrogel capsules encapsulating live mammalian cells, combining the population of capsules with a pharmaceutically acceptable aqueous solution described herein and placing a desired volume of the composition into a biocompatible, sealable container in a manner that produces a capsule layer in which substantially all of the capsules in the

composition volume are distributed substantially uniformly across the bottom of the container at a depth equal to about 1.00 to about 1.25 times the mean diameter of the capsules in the composition. In an example, the method further comprises adding a desired volume of the pharmaceutically acceptable solution in a manner to form a solution layer on top of the capsule layer, and sealing the container. In an example, the method further comprises storing the sealed container for a desired time period at a temperature of 2˚C to 30˚C or about 12˚C to 30˚C (e.g., about 15-25˚C). for a desired time period and assessing the viability of the encapsulated cells in the composition at one or more time points during the desired time period.

In a still further example, the present disclosure features the use of a hydrogel capsule composition described herein for treating a mammalian subject (e.g., a human subject) in need of treatment with a substance (e.g., a protein) produced by the encapsulated cells in the composition. In an example, the subject is treated by a method comprising administering a desired amount of the composition to the subject, e.g., by implanting the desired amount into the intraperitoneal cavity of the subject. In an example, the method comprises providing the hydrogel capsule composition in a sealed container described herein, unsealing the container, and removing the desired quantity. In an example, the encapsulated cells in the hydrogel capsule composition are genetically modified to produce one of the exogenous proteins described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a graph of viability of cells encapsulated in alginate hydrogel spheres and stored for up to three days in an aqueous solution that lacked calcium or in the same solution with added calcium, with the error bars representing the standard deviation (SD) between two replicates per time point.

FIG. 2 shows a graph of plasma FVIII levels in mice implanted with alginate hydrogel spheres encapsulating FVIII-expressing cells after the spheres had been stored in different storage solutions, with the error bars representing the standard deviation (SD) between four mice per storage condition.

FIGS. 3A and 3B show graphs of plasma FVIII levels in mice implanted with alginate hydrogel spheres encapsulating FVIII-expressing cells after the spheres had been stored in different storage solutions for the indicated time period, with the error bars representing the standard deviation (SD) between four mice per storage condition.

CLAIMS

1. A composition comprising a population of hydrogel capsules disposed in a pharmaceutically acceptable aqueous solution, wherein each hydrogel capsule in the population encapsulates a plurality of live mammalian cells, wherein the solution has a pH of between 6.0 and 9.0 at 12°C to 30°C (e.g., about 15-25°C), and comprises a calcium salt at an elemental calcium concentration of between about 1.0 millimolar (mM) and about 10 mM.

2. The composition of claim 1, wherein:

   (i) the solution has an osmolality of about 250 milliosmole/kg solution to about 350 milliosmole/kg solution; and/or

   (ii) the pH of the solution is between 6.5 and 9.0 at 15 °C to 25 °C.

3. The composition of claim 1, wherein each hydrogel capsule in the population comprises an ionically cross-linked alginate.

4. The composition of claim 1, wherein the elemental calcium concentration is between an x value and a y value, wherein the x and y values are selected from the group consisting of:
   (i) x = about 1.1 mM and y = about 8.0 mM, 6.0 mM, 4.0 mM, or 2.0 mM;
   (ii) x = about 1.2 mM and y = about 5 mM, 4 mM, 3 mM, or 2.0 mM;
   (iii) x = about 1.2 mM and y = about 2.0 mM or 1.5 mM;
   (iv) x = about 1.3 mM and y = about 1.4 mM;
   (v) x = about 1.4 mM and y = about 4.0. mM, 3.0 mM, or 2.0 mM; and
   (vi) x = about 1.5 mM and y = about 2.5 mM.

5. The composition of claim 1, wherein the solution further comprises at least one carbon source (e.g., a sugar (e.g., dextrose, glucose, galactose, hexose, fructose, maltose), glycerol, glutamine, pyruvate or salt thereof).

6. The composition of claim 1, wherein the solution further comprises:

(a) a buffering agent comprising one or more of an acetate salt (e.g., sodium acetate), a gluconate salt (e.g., sodium gluconate), a phosphate salt (e.g., sodium phosphate monobasic), a bicarbonate salt (e.g., sodium bicarbonate), and a lactate salt (e.g., sodium lactate); or
(b) a buffering agent comprising sodium acetate and sodium gluconate; or,
(c) a buffering agent consisting essentially of about 0.5-5 g/L sodium acetate (e.g., 2 g/L, e.g., 2.29 g/L sodium acetate) and about 0.5-10 g/L sodium gluconate (e.g., 5 g/L, e.g., 5.18 g/L).

7. The composition of claim 5, wherein the carbon source is glucose, and the solution does not contain any added glutamine or phenol red.

8. The composition of claim 6, wherein the buffering agent comprises sodium bicarbonate and sodium phosphate and the solution does not contain any added HEPES or sodium pyruvate.

9. The composition of claim 1, wherein the calcium salt is calcium chloride.

10. The composition of claim 1, wherein the solution comprises about 1.5 mM to 2.5 mM calcium chloride, about 5 mM to about 25 mM D-glucose, and about 40 mM to about 50 mM sodium bicarbonate.

11. The composition of claim 1, wherein the solution further comprises:
    (i) a magnesium compound (e.g., magnesium chloride or magnesium sulfate);
    (ii) a potassium compound (e.g., potassium chloride);
    (iii) sodium chloride;
    (iv) a set of amino acids, which comprises histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine;
    (v) a set of amino acids which comprises arginine, glycine, cystine, serine, and tyrosine and the set of vitamins also comprises choline or a choline salt, a vitamin B5 compound, a folate compound, riboflavin and i-inositol; and/or
    (vi) a set of vitamins, which comprises a vitamin B1 compound (e.g., thiamine or a thiamine salt, e.g., thiamine hydrochloride), a vitamin B3 compound (e.g., nicotinic acid or niacinamide) and a vitamin B6 compound (e.g., pyroxidine or a pyroxidine salt, e.g., pyroxidine hydrochloride).

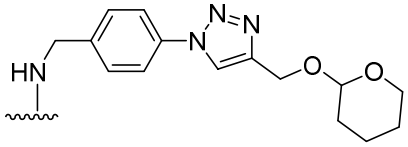
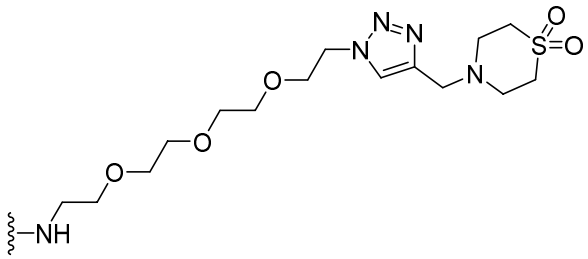
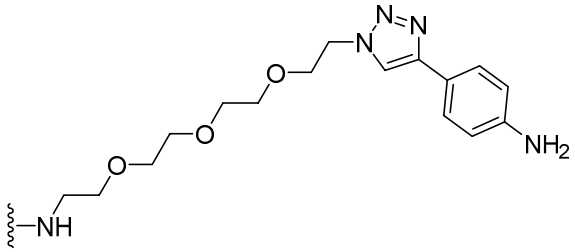
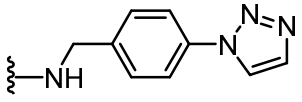
12. The composition of claim 1, wherein each hydrogel capsule in the population has a sphere-like or spherical shape and comprises:

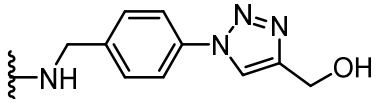
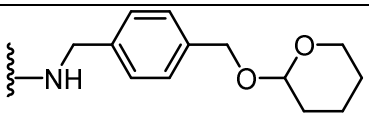
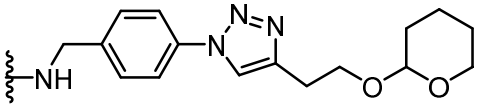
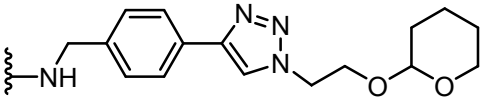
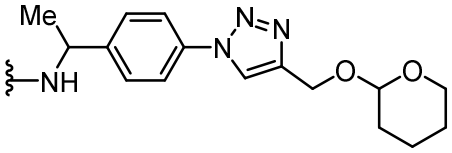
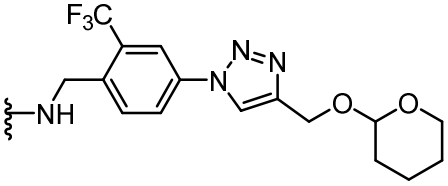
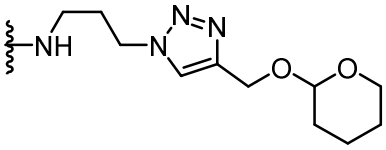
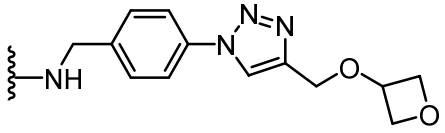
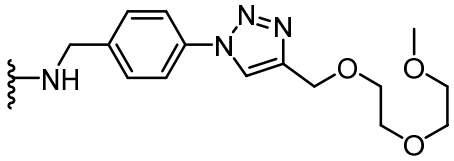
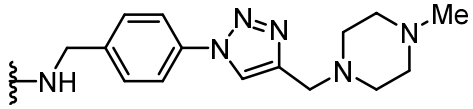
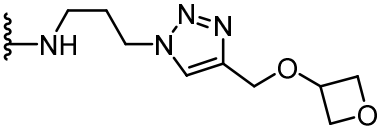
(a) a cell-containing compartment which comprises the plurality of live cells encapsulated in a first polymer composition; and

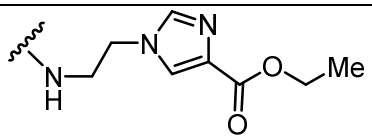
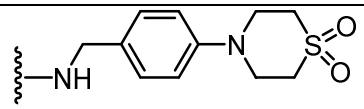
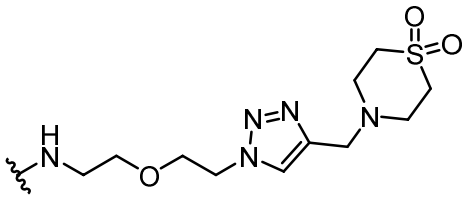
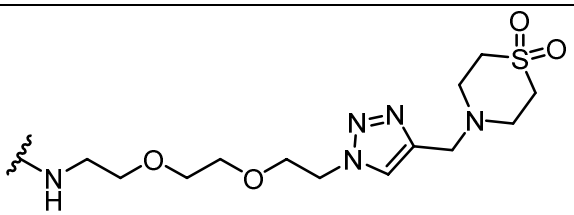
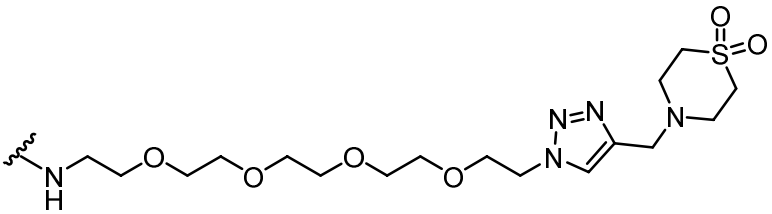
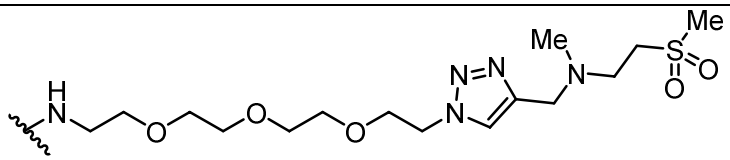
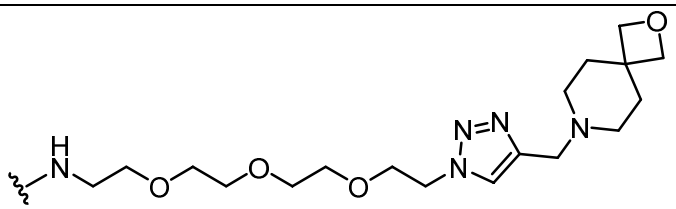
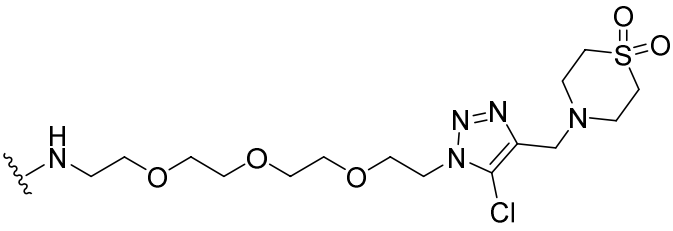
(b) a barrier compartment surrounding the cell-containing compartment and comprising a second polymer composition which comprises the ionically cross-linked alginate;

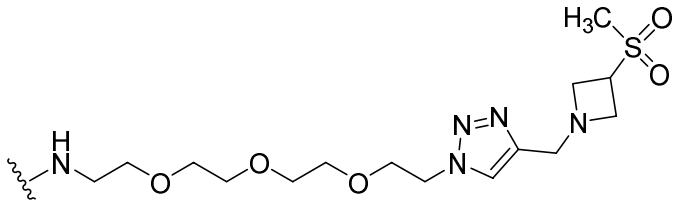
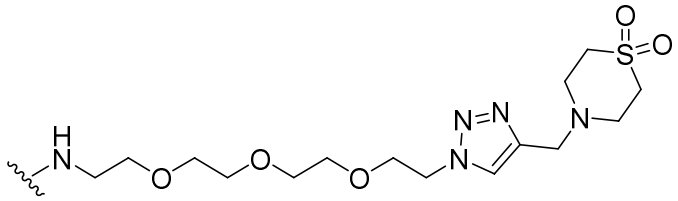
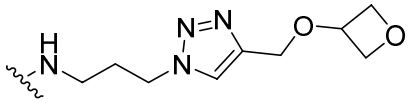
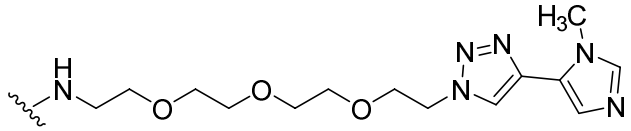
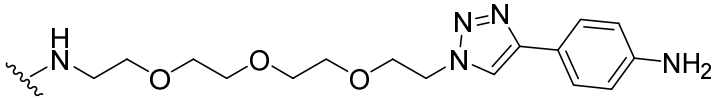
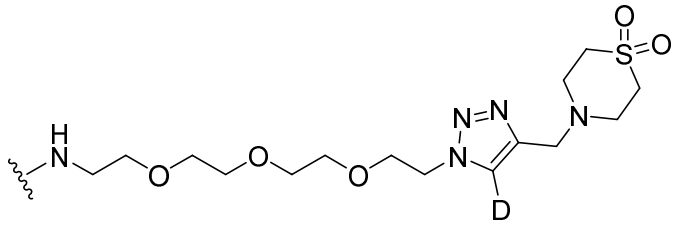
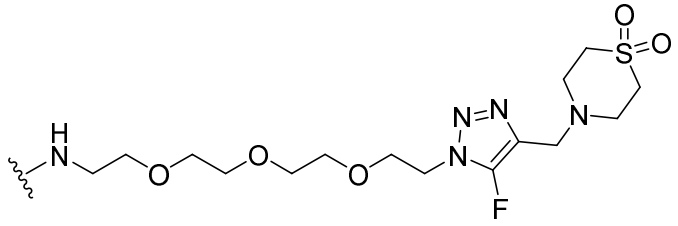
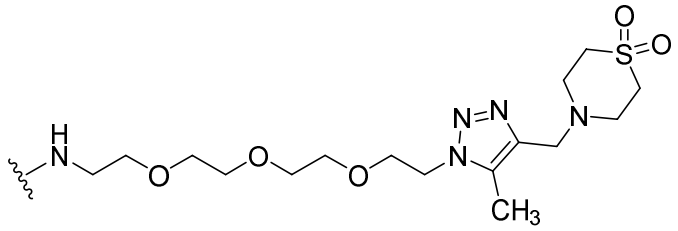
wherein the mean diameter of the hydrogel capsules in the population is about 500 micrometers (μm) to about 5000 μm, about 1000 (μm) to about 3000 μm, about 1100 μm to about 2500 μm, about 1200 μm to about 2300 μm, about 1300 μm to about 2100 μm, about 1400 μm to about 2000 μm, about 1400 μm to about 1900 μm, about 1400 μm to about 1800 μm.

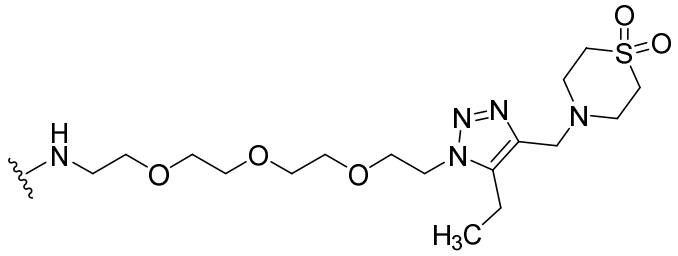
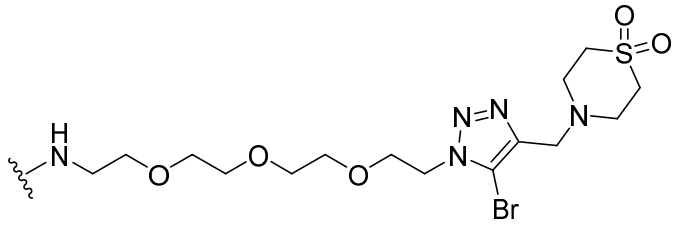
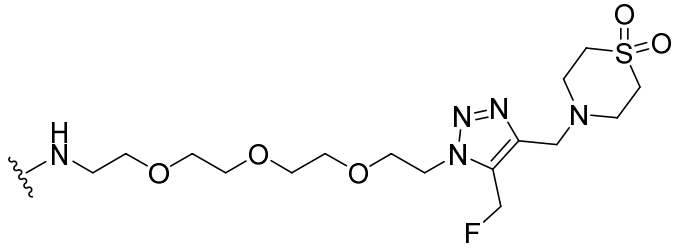
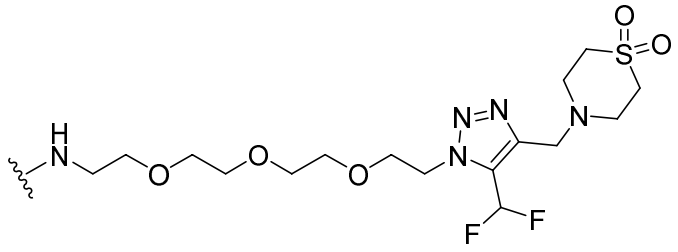
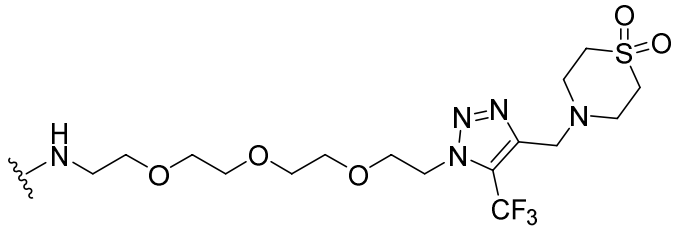
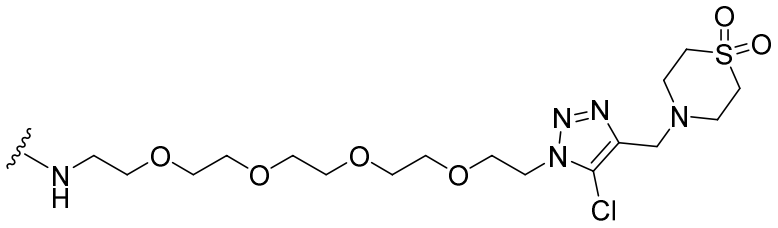
13. The composition of claim 12, wherein the first polymer composition comprises an alginate covalently modified with a cell-contacting peptide via a linker and wherein the cross-linked alginate in the barrier compartment comprises an alginate covalently modified with at least one afibrotic compound, optionally a compound selected from:

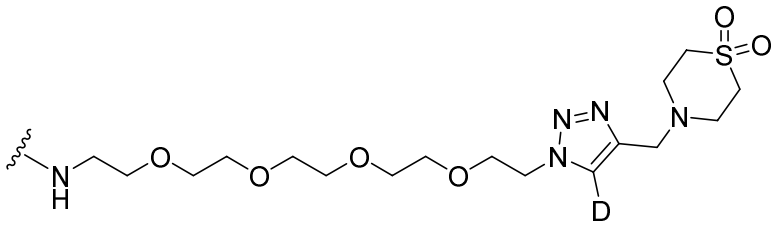
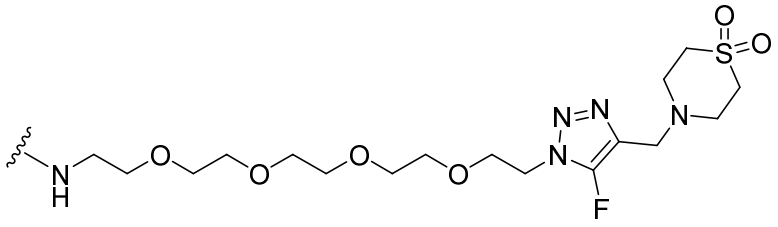
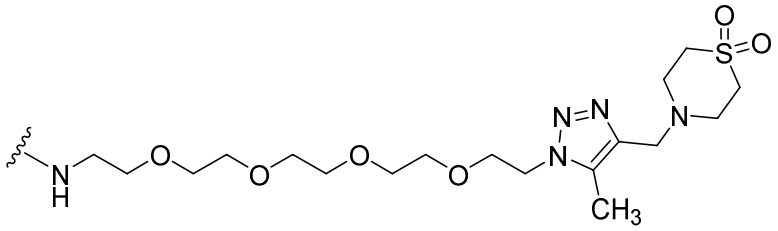
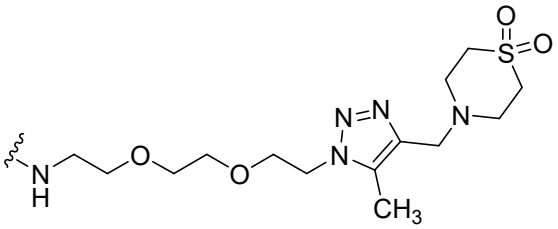
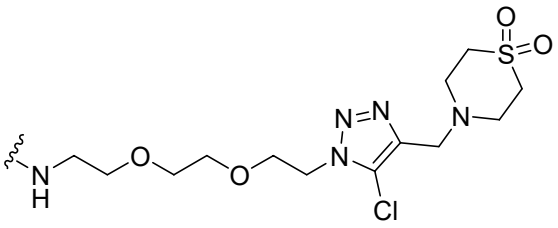
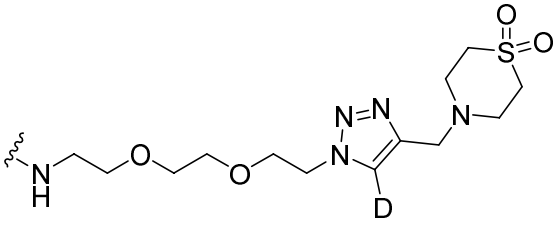
Compound No.	**Structure**
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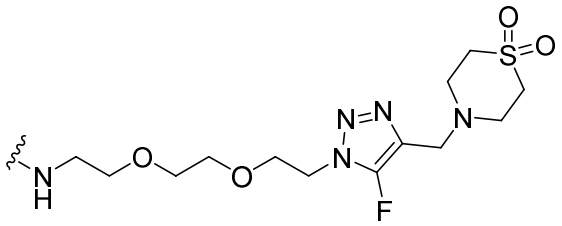
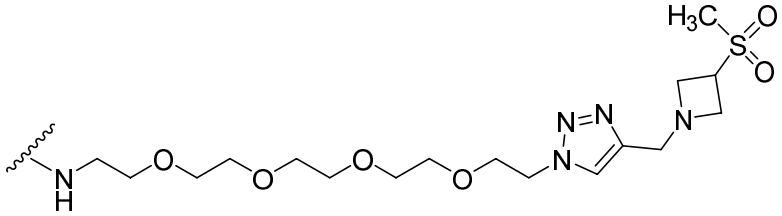
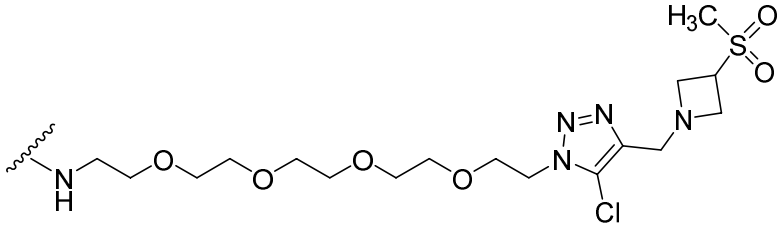
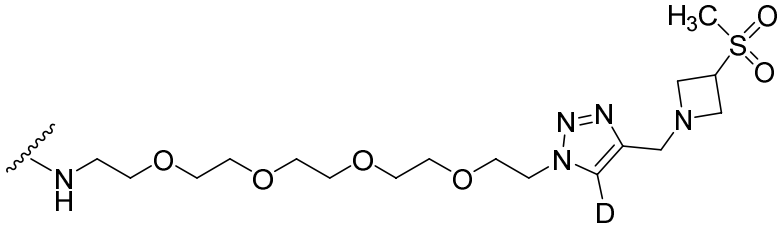
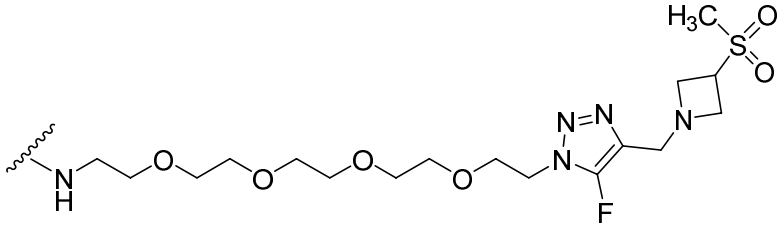
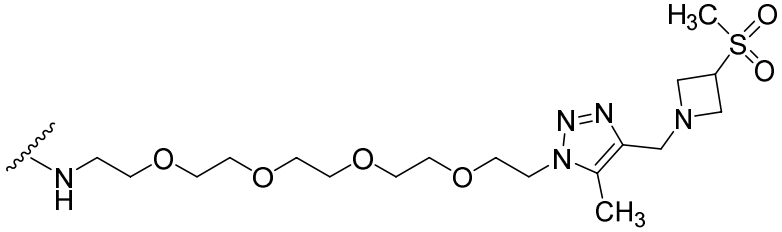
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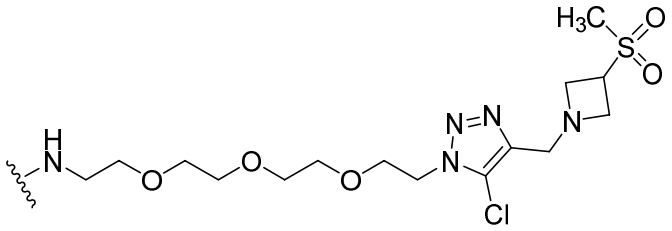
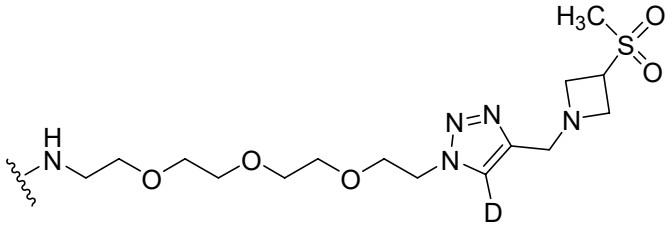
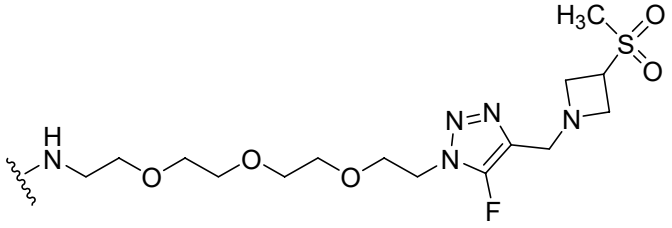
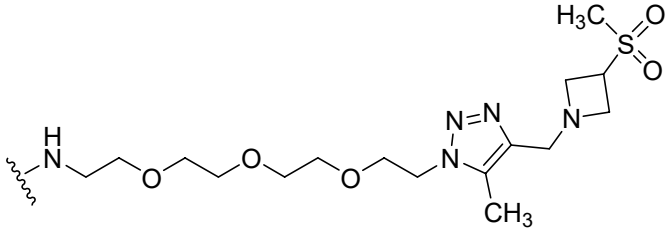
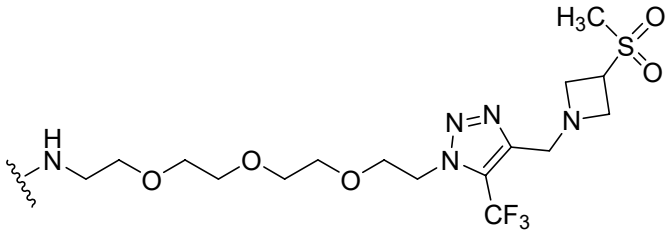
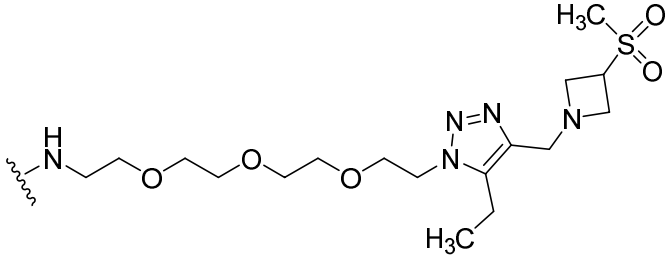
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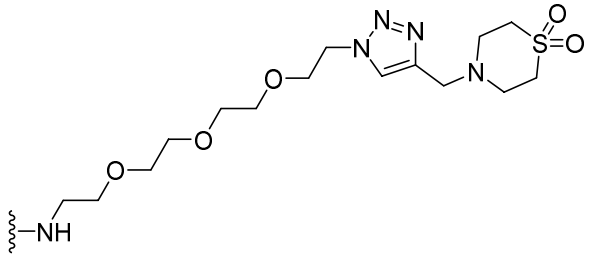
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14. The composition of claim 13, wherein the ionically cross-linked alginate in the barrier compartment comprises:
    (a) barium ions as at least one cross-linking agent; and/or,
    (b) a mixture of the covalently modified alginate and an unmodified alginate.

15. The composition of claim 14, wherein the covalently modified alginate in the cell-containing compartment is ionically cross-linked with barium ions as at least one cross-linking agent.

16. The composition of claim 13, wherein:
    (a) the mean diameter of the hydrogel capsules in the population is 1400 µm to 2000 µm, or1400 µm to 1600 µm, or 1000 µm to 1200 µm;
    (b) the alginate in the first polymer composition has a molecular weight of 150 to 250 kDa and a G:M ratio of greater than or equal to 1.5;
    (c) the cell-contacting peptide consists of RGDSP and the linker is a single glycine residue attached to the N-terminus of the cell-contacting peptide;
    (d) the alginate in the covalently-modified alginate in the barrier compartment has a molecular weight of <75 kDa and a G:M ratio of greater than or equal to 1.5;
    (e) the afibrotic compound is



; and

    (f) the unmodified alginate in the barrier compartment has a molecular weight of 150 kDa to 250 kDa and a G:M ratio of greater than or equal to 1.5.

17. The composition of claim 1, wherein the live mammalian cells are:
    (a) human cells;
    (b) derived from an induced pluripotent stem cell; or,
    (c) derived from an RPE cell.

18. The composition of claim 1, wherein the encapsulated cells comprise:

(a) single cells;

(b) one or more cell clusters; and/or,

(c) cells disposed on a microbead.

19. The composition of claim 1, wherein the live mammalian cells are genetically modified to express and secrete a therapeutic substance, e.g., a therapeutic polypeptide.

20. The composition of claim 1, wherein the mammalian cells comprise an exogenous nucleotide sequence which encodes a therapeutic polypeptide, optionally wherein the therapeutic polypeptide is a growth factor, a blood coagulation factor, an enzyme, a cytokine, a cytokine receptor, an antibody or antigen-binding fragment thereof.

21. The composition of claim 20, wherein the therapeutic polypeptide is selected from:

(a) an FVIII protein, a FIX protein, or a FVII protein; or,

(b) a GLA protein, an IDUA protein, an IDS protein, an ARSB protein, or a GBA protein.

22. The composition of claim 12, wherein the plurality of live mammalian cells is between about 5,000 to about 250,000 cells, about 10,000 to about 125,000 cells, about 20,000 to about 75,000 cells, about 12,500 to about 40,000 cells, or about 15,000 to about 30,000 cells.

23. The composition of claim 1, which comprises about 200 to about 400 of the hydrogel capsules per milliliter of the pharmaceutically acceptable solution.

24. A sealed container comprising the composition of any one of the above claims.

25. The sealed container of claim 24, wherein:

(a) the volume of the aqueous solution (VS) in the container is about equal to or greater than the volume of the hydrogel capsules (VHC) in the container; or,

(b) the ratio of VS to VHC is selected from the group consisting of:

(i) between about 1.5 and about 100,

(ii) between about 2 and about 75,

(iii) between about 3 and about 50,

(iv) between about 4 and about 40,

(v) between about 5 and about 30, and

(vi) between about 10 and about 20.

26. The sealed container of claim 25, which is configured to be stored in a manner to allow substantially all of the hydrogel capsules in the composition to be distributed substantially uniformly across the bottom interior surface of the stored container in a capsule layer with a depth equal to about 1.00 to about 1.25 times the mean diameter of the capsules in the composition.

27. The sealed container of claim 25, wherein all interior surfaces of the container consist essentially of fluorinated ethylene propylene (FEP) or polyethylene terephthalate glycol (PETG).

28. The sealed container of claim 25, which is a flexible, rectangular bag which comprises a first port configured to allow addition of the composition to the bag and a second port configured to allow removal of a desired volume of the composition from the bag.

29. The sealed container of claim 25, wherein the mean diameter of the hydrogel capsules in the container is about 1500 μm and the ratio of VHC to VS is at least about 1:1, 1:2, 1:3 and less than about 1:40, e.g., about any of 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, and 1:35.

30. Use of a composition of claim 1 in the manufacture of a medicament for treating a subject in need of a therapeutic substance, wherein a therapeutically effective amount of the composition is to be administered to the subject, wherein the subject is human, and wherein the administration comprises the effective amount to be disposed into the intraperitoneal cavity of the subject.

31. A method of making a sealed container comprising a hydrogel capsule composition, wherein the method comprises:

(i) providing a population of hydrogel capsules encapsulating live mammalian cells, optionally wherein the mammalian cells are genetically modified to express and secrete a therapeutic substance, e.g., a therapeutic polypeptide;

(ii) combining the population of hydrogel capsules with a pharmaceutically acceptable

aqueous solution;

(iii) placing a desired volume of the capsule composition into a biocompatible, sealable container in a manner that produces a capsule layer in which substantially all of the capsules in the composition volume are distributed substantially uniformly across the bottom of the container at a depth equal to about 1.00 to about 1.25 times the mean diameter of the capsules in the composition; and

(iv) sealing the container.

32. The method of claim 31, wherein the aqueous solution has a pH of between 6.0 and 9.0 at 12°C to 30°C (e.g., about 15-25°C), and comprises a calcium salt at an elemental calcium concentration of between about 1.0 millimolar (mM) and about 10 mM.